

UNIVERSITY OF FLORIDA BIOSAFETY MANUAL



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Emergency Telephone Numbers

	Normal Business Hours	Evenings/Weekends
Fire/Police/Medical	911	911
University Police Department	352-392-1111	352-392-1111
Environmental Health & Safety	352-392-1591	352-392-1111
Biological Spill (Campus)	352-392-1591	352-392-1111
Chemical Spill (Campus)	352-392-1591	352-392-1111
Needlestick Hotline	352-265-2727	352-265-2727
Pest Control	352-392-3410 or 352-392-1591	352-392-1111
Radiation Spill (Campus)	352-392-7359 or 352-392-1591	352-392-1111
Workers' Compensation	352-392-4940	800-455-2079
Florida Poison Information Center	800-222-1222	800-222-1222

Introduction

The University of Florida (UF) strives to provide a safe working and learning environment for everyone on campus. The Biosafety Manual serves to familiarize UF faculty, staff, students, volunteers and visitors with the local, state and federal policies and procedures for the safe handling of biohazardous materials. Compliance with these policies and procedures minimizes the risk of occupational exposure to and accidental release of biohazardous materials.

The primary responsibility for ensuring safe conduct and conditions in the laboratory or research area resides with the principal investigator (PI). The PI shall be familiar with the contents of this manual, make certain their staff are familiar with it, and ensure all work with biohazardous materials is conducted in compliance with University policies and procedures. The UF Biosafety Manual shall be used in conjunction with the [UF Laboratory Safety Manual](#) which provides additional general safety information.

Responsibilities

All individuals handling or manipulating biohazardous materials shall be committed to safety and must demonstrate the ability to understand and follow:

1. Safe work practices as detailed in this manual, the [UF Laboratory Safety Manual](#) and accompanying [EH&S policies and guidelines](#).
2. Applicable local, state and federal requirements pertaining to work with these materials.

Environmental Health & Safety Biosafety Office

1. Provide guidance, information and training regarding biosafety programs at UF.
2. Review and track biohazard project registrations.
3. Perform annual safety surveys for laboratories working with biohazards.
4. Evaluate work practices, safety and personal protective equipment and facilities used for research activities utilizing biohazards.
5. Develop and implement administrative controls, in conjunction with the [Institutional Biosafety Committee \(IBC\)](#) when appropriate, for biohazards.
6. Serve as the liaison with local, state and federal regulatory agencies (i.e. Florida Department of Health, CDC, USDA).

Institutional Biosafety Committee (IBC)

1. Review and approve experiments utilizing recombinant/synthetic nucleic acids as required by the NIH Guidelines and those that are conducted at biosafety level 3 (BSL-3).
2. Works in conjunction with the UF Biosafety Office to establish, monitor and enforce policies or procedures for work with biohazardous materials.

Principal Investigator (PI)

1. [Register](#) (and update) experiments using biohazardous material with the Biosafety Office and/or [Institutional Biosafety Committee](#).
2. Complete all required [EH&S training](#) triggered by the laboratory's risk assessment.
3. Adhere to all local, state and federal requirements applicable to his/her research.
4. Ensure that all members of the laboratory are familiar with and adhere to safe research practices.
5. Train all laboratory staff and visitors on the specific hazards that may be encountered in the laboratory and provide information on how to minimize exposure to such hazards.
6. Promptly [report](#) exposures to or releases of biohazardous materials.

Laboratory Personnel (employees, students, volunteers, etc.)

1. Complete all required [EH&S training](#) triggered by the laboratory's risk assessment.
2. Be familiar with and follow all protocols and procedures for the use, storage and disposal of biohazardous material.
3. Be familiar with and use required personal protective equipment (PPE).
4. Promptly [report](#) exposures to or releases of biohazardous materials.

Biological Hazards

A biological hazard, or biohazard, refers to any biological material that presents a risk or potential risk to the health of humans, animals, or the environment. Biological hazards include pathogenic agents such as bacteria, viruses, fungi, and parasites; recombinant and synthetic nucleic acid molecules as defined by the [NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules \(NIH Guidelines\)](#); [acute biological toxins](#) (those with an $LD_{50} \leq 100 \mu\text{g/kg}$ body weight); human blood/tissues/organs/cells and certain human body fluids.

Regulatory Oversight of Biohazards

[NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules \(NIH Guidelines\)](#)

The purpose of the NIH Guidelines is to specify the biosafety practices and containment principles for constructing and handling: (i) recombinant nucleic acid molecules, (ii) synthetic nucleic acid molecules, including those that are chemically or otherwise modified but can base pair with naturally occurring nucleic acid molecules, and (iii) cells, organisms and viruses containing such molecules. The NIH Guidelines apply to all research that is conducted at or sponsored by any institution that receives any support for recombinant or synthetic nucleic acid research from NIH. Since UF receives funding from NIH, compliance with the NIH Guidelines is mandatory for all PIs at UF conducting research covered under the Guidelines. It is the PIs responsibility to ensure that his/her laboratory complies with the NIH Guidelines. See the [NIH Guidelines Flowchart](#) for a summary of experiments covered under the

Guidelines. The [Viral Vector Fact Sheet](#) provides information on commonly used viral vectors – work with these vectors falls under the purview of the NIH Guidelines.

[CDC/NIH Biosafety in Microbiological and Biomedical Laboratories \(BMBL, 6th edition\)](#)

While the BMBL is not meant to be a regulatory document, it has served as the cornerstone of biosafety practice in the U.S. since the first edition was published in 1984 and outlines best practices for the safe conduct of work with biological materials. The principles of biosafety discussed in the BMBL are containment and risk assessment. The fundamentals of containment include the microbiological practices, safety equipment, and facility safeguards that protect laboratory workers, the environment and the public from exposure to infectious microorganisms that are handled and stored in the laboratory. Risk assessment is the process that enables the appropriate selection of microbiological practices, safety equipment and facility safeguards that can help prevent or minimize exposure to infectious microorganisms handled in the laboratory. The University of Florida is committed to following the BMBL standards as a minimum for biological safety.

[Florida Department of Health Biomedical Waste Program](#)

The Florida Department of Health sets forth requirements for the safe handling and treatment of biomedical waste in [Chapter 64E-16](#), of the Florida Administrative Code. All facilities that generate, transport, store or treat biomedical waste must comply with the regulations in order to protect public health. The regulations prescribe minimum sanitary practices relating to the management of biomedical waste, including segregation, handling, labeling, storage, transport and treatment.

Under Chapter 64E-16, biomedical waste training is required for all personnel whose responsibilities include some aspect of managing biomedical waste. This training (available in [MyTraining](#) – UF_EHS851_OLT) is required prior to assuming any duties associated with biomedical waste and annually thereafter.

[OSHA Bloodborne Pathogens Standard](#)

OSHA's Bloodborne Pathogens (BBP) Standard (29CFR 1910.1030) is a federal regulation that prescribes safeguards to protect workers against health hazards related to bloodborne pathogens. UF follows the OSHA standard and requires all individuals with occupational exposure to human blood or other potentially infectious materials (OPIM) as defined in the BBP standard to participate in the BBP program. Specific information related to the UF BBP program can be found [here](#).

[Federal Select Agent Program](#)

The Federal Select Agent Program, jointly comprised of the Centers for Disease Control Division of Select Agents and Toxins (DSAT) and the USDA Animal and Plant Health Inspection Service (APHIS) Division of Agricultural Select Agents and Toxins (DASAT), oversees the possession, use and transfer of biological select agents and toxins, which have the potential to pose a severe threat to public, animal or plant health or to animal or plant products. Select agent possession, use and transfer is strictly regulated and failure to comply with federal regulations is punishable by both fines and imprisonment. Click [here](#) to see the current list of select agents.

If you are considering work with any select agents or toxins, contact the [Biosafety Office](#) to discuss the feasibility of the proposed work. All registration activities for these agents must be coordinated through the Biosafety Office; researchers cannot register directly with the USDA or CDC. Plan accordingly! Approval can take 6 months or more to complete.

United States Government Policy for Institutional Oversight of Life Sciences Dual Use Research of Concern (DURC)

Dual Use Research of Concern (DURC) is life sciences research that, based on current understanding, can be reasonably anticipated to provide knowledge, information, products, or technologies that could be directly misapplied to pose a significant threat with broad potential consequences to public health and safety, agricultural crops and other plants, animals, the environment, materiel, or national security. This policy communicates the practices and procedures required to ensure that dual use research of concern is identified at the institutional level and risk mitigation measures are implemented as necessary.

UF, as an institution that receives United States Government funding for life sciences research, must adhere to the oversight requirements specified in the policy. Additional information about DURC research can be found [here](#) along with the [University of Florida Policy on Dual Use Research of Concern](#).

Risk Groups and Biosafety Levels

Risk Groups

Biological agents can be classified into Risk Groups (RG) according to the relative hazard they pose with 1 being the lowest and 4 being the highest. Hazardous characteristics considered in the risk group classification include the agent's ability to infect and cause disease in a susceptible human or animal host, virulence, and availability of preventive measures and effective treatments for the disease. Both the [NIH Guidelines](#) and the [World Health Organization \(WHO\) Laboratory Biosafety Manual \(3rd edition\)](#) describe four different risk groups for biological agents. **Table 1** shows the risk group classifications for each organization.

Table 1. Risk Group Classifications According to NIH and WHO

Risk Group Classification	NIH Guidelines	WHO Laboratory Biosafety Manual	Examples
Risk Group 1 (RG1)	Agents that are not associated with disease in healthy adult humans	A microorganism that is unlikely to cause human or animal disease. No or low individual and community risk.	<i>Bacillus subtilis</i> , nonpathogenic K-12 strains of <i>E. coli</i> , Adeno-associated virus
Risk Group 2 (RG2)	Agents that are associated with human disease which is rarely serious and for which preventative or therapeutic interventions are <i>often</i> available	A pathogen that can cause human or animal disease but is unlikely to be a serious hazard to laboratory personnel, the community, livestock or the environment. Laboratory exposures may cause serious infection, but effective treatment and preventative measure are available, and the risk of infection is limited. Moderate individual risk but low community risk.	Hepatitis B virus, <i>Vibrio cholerae</i> , <i>Staphylococcus aureus</i>

Risk Group 3 (RG3)	Agents that are associated with serious or lethal human disease for which preventative or therapeutic interventions <i>may</i> be available (high individual risk but low community risk)	A pathogen that usually causes serious human or animal disease but does not ordinarily spread from one infected individual to another. Effective treatment and preventative measures are available. High individual risk but low community risk.	<i>Brucella abortus</i> , Chikungunya virus
Risk Group 4 (RG4)	Agents that are likely to cause serious or lethal human disease for which preventative or therapeutic interventions are <i>not usually</i> available (high individual risk and high community risk)	A pathogen that usually causes serious human or animal disease and that can be readily transmitted from one individual to another, directly or indirectly. Effective treatment and preventative measures are not usually available. High individual risk and high community risk.	Ebola virus, Lassa virus

Biosafety Levels (BSL)

In contrast to Risk Groups, Biosafety Levels (BSL) prescribe combinations of laboratory practices, safety equipment and laboratory facilities which are designed to minimize exposure of personnel and the environment to infectious agents. Like Risk Groups, Biosafety Levels are graded from 1 through 4, with BSL-1 representing a basic level of containment that relies primarily on the use of standard microbiological practices while BSL-4 is the maximum level of containment and is usually reserved for work with dangerous and exotic microorganisms. It is important to note that the Risk Group a biological agent is assigned to is only one factor to consider when determining the biosafety level and an agent's Risk Group will not always be synonymous with the required Biosafety Level for an agent (i.e. a RG2 agent may require BSL-3 containment).

The essential elements of all four Biosafety Levels is the use of standard microbiological practices. The American Biological Safety Association International (ABSA) has an excellent list of these practices: [Principles of Good Microbiological Practice](#). The [CDC/NIH Biosafety in Microbiological and Biomedical Laboratories \(BMBL 6th edition\)](#) details the requirements for the four different biosafety levels. A summary of the different Biosafety Levels is detailed in **Table 2**.

Table 2. Summary of Laboratory Biosafety Levels (BSLs) adapted from BMBL 6th edition

BSL	Agents	Special Practices	Primary Barriers and Safety Equipment	Facilities (Secondary Barriers)
1	Well-characterized agents not known to consistently cause disease in immunocompetent adult humans and present minimal potential hazard to laboratory personnel and the environment	Standard microbiological practices	No primary barriers required PPE: lab coats and gloves; protective face, protective eyewear	Laboratory doors; sink for handwashing; laboratory bench; eyewash station readily available; windows that open fitted with screens; lighting adequate for all activities
2	Agents associated with human disease and pose moderate hazards to personnel and the environment	BSL-1 plus: Limited access; occupational medical services (medical evaluation, surveillance and treatment) as appropriate; all procedures that may generate aerosol or splash conducted in a biosafety cabinet (BSC); decontamination process needed for laboratory equipment	BSCs or other primary containment device used for manipulations of agents that may cause splashes or aerosols PPE: lab coats and gloves; protective face, eyewear, respiratory protection as needed	BSL-1 plus: Self-closing doors; autoclave available
3	Indigenous or exotic agents that may cause serious or potentially lethal disease through the inhalation route of exposure	BSL-2 plus: Access limited to those with need to enter; viable material removed from laboratory in primary and secondary containers; opened only in BSL-3 or ABSL-3 laboratories; all procedures with infectious materials performed in a BSC	BSCs for all procedures with viable agents PPE: solid front gowns, scrubs or coveralls; two pairs of gloves; protective face, eyewear, respiratory protection as needed	BSL-2 plus: Physical separation from access corridors; access through two consecutive self-closing doors; hands-free sink; sealed windows; ducted air ventilation system with negative airflow into the lab; seams, floors, walls, and ceiling surfaces are sealed and spaces around doors and ventilation openings are capable

				of being sealed to facilitate space decontamination.
4	Dangerous and exotic agents that pose high individual risk of aerosol-transmitted laboratory infections and life-threatening disease that are frequently fatal, for which there are no vaccines or treatments; and related agents with unknown risk of transmission	BSL-3 plus: Clothing change before entry; daily inspections of essential containment and life support systems; all wastes decontaminated prior to removal from laboratory; shower on exit	BSCs for all procedures with viable agents PPE: for cabinet laboratories: same as BSL-3; for suit laboratories: full-body, air-supplied, positive-pressure suit	BSL-3 plus: Entry through airlock with airtight doors; walls, floors, ceilings form sealed internal shell; dedicated non-recirculating ventilation system required; double-door pass-through autoclave required

Animal Biosafety Levels (ABSL)

There are four biosafety levels that describe combinations of practices, safety equipment and facilities for experiments with animals involved in infectious disease research and other animal studies that require containment such as those with animals exposed to recombinant/synthetic nucleic acids. Like biosafety levels, the four levels (ABSL-1 through ABSL-4) provide increasing levels of protection to personnel and the environment. The [CDC/NIH Biosafety in Microbiological and Biomedical Laboratories \(6th edition\)](#) details the requirements for the four different animal biosafety levels.

Plant Biosafety Levels (PBSL)

Research involving plants and plant-associated microbes (fungi, bacteria, viruses) and macroorganisms (arthropods and nematodes) generally does not pose a risk to human health but can pose a hazard to other plants and the environment. Plant biosafety levels are designated with a “P” after the containment level (i.e. BSL-1P) and like biosafety and animal biosafety levels, there are four levels of plant containment. The [NIH Guidelines](#) specify physical and biological containment conditions and practices suitable to the greenhouse conduct of experiments involving recombinant or synthetic nucleic acid molecule-containing plants, plant-associated microorganisms and small animals in [Appendix L](#) of the NIH Guidelines. When plants are grown in a laboratory, physical containment as described in [Appendix G](#) of the NIH Guidelines applies. Additional guidance for plant biosafety practices can be found in “[A practical Guide to Containment: Plant Biosafety in Research Greenhouses](#)” and “[Containment Facilities and Safeguards for Exotic Plant Pathogens and Pests](#)”

Arthropod Containment Levels (ACL)

The American Committee of Medical Entomology publishes the [Arthropod Containment Guidelines](#) which supplements the [CDC/NIH Biosafety in Microbiological and Biomedical Laboratories \(6th edition\)](#) and can be used as a reference for research laboratories to assess risk and establish protocols for the safe handling of arthropod vectors of human and animal disease agents. Additional information on working with arthropods at UF can be found on the [Research Involving Flying Insects](#) page.

Risk Assessment

Both the [WHO Laboratory Biosafety Manual \(4th edition\)](#) and the [BMBL 6th edition](#) recognize the importance of specific risk assessment to determine both the hazards of the work and the mitigation necessary to reduce the risk to an acceptable level. A risk- and evidence-based approach to biosafety allows safety measures to be balanced with the actual risk of working with biological agents on a case-by-case basis as it takes into consideration not only the risk group of an agent but the procedures being performed and the personnel performing the procedures.

In order to minimize the risks of working with biological hazards and provide a safe work environment at the University of Florida, a risk assessment shall be conducted to determine the appropriate work practices and containment requirements. Proper risk assessments will consider the following:

- Hazards associated with the biological material
 - Pathogenicity
 - Virulence
 - Host range
 - Communicability
 - Mode of transmission
 - Environmental stability

- Activities that might result in exposure to, or release of, the biological hazard
 - Aerosol-generating procedures
 - Use of sharps
 - Animal studies
 - Laboratory vs field studies
 - Use of high concentrations and/or large volumes of material
- Laboratory personnel factors
 - Education and training
 - Experience
 - Health status
 - Motivation, attentiveness

Risk assessments shall be conducted when planning a new research project and any time significant changes are made to a research project. Significant changes include new personnel, new hazards, new procedures/techniques, new equipment, new location of research. Use the [Biological Hazard Assessment and Risk Mitigation](#) template to aid in conducting a risk assessment.

Biohazard Project Registration

The purpose of the [Biohazard Project Registration](#) process is to identify and assess risks associated with handling certain biological materials so that appropriate mitigations can be put in place to minimize exposure to, or release of, such materials. Project approvals will stipulate the requirements for the appropriate containment levels, procedures, and practices needed to safely work with these materials.

The following requires registration and approval from the Biosafety Office/Institutional Biosafety Committee prior to beginning work:

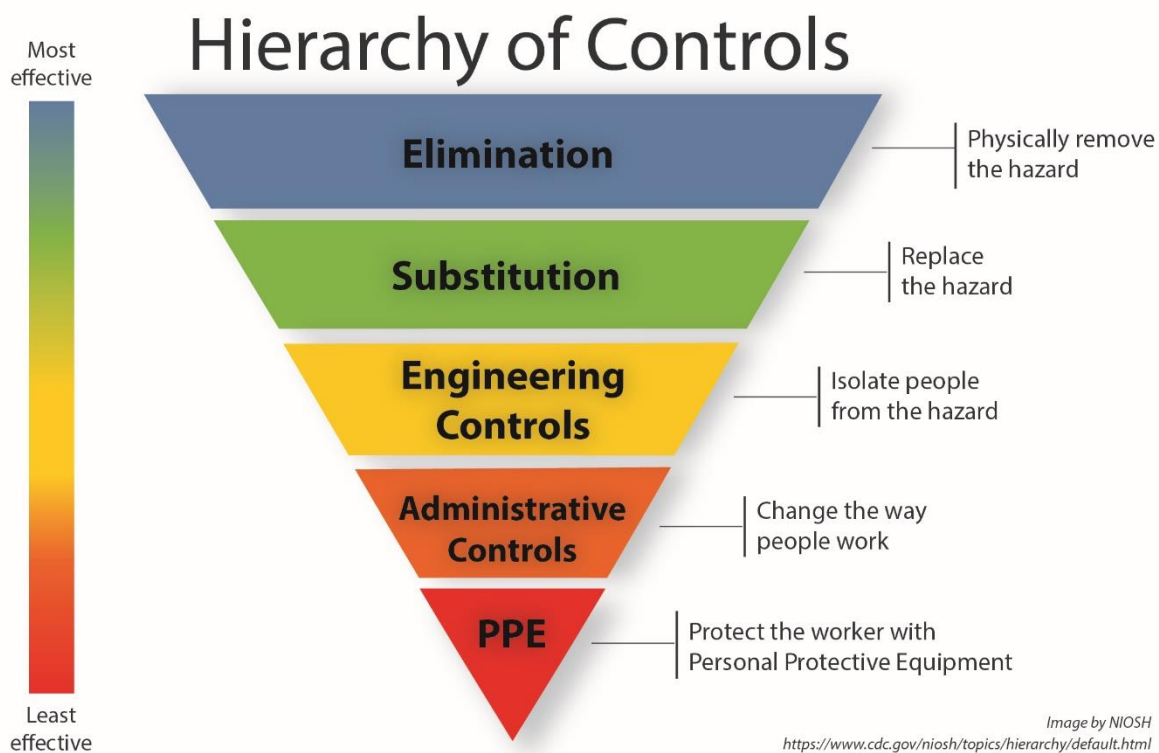
- Use of Risk Group 2 or Risk Group 3 infectious biological agents pathogenic to otherwise healthy humans
- Analysis of samples known or highly suspected to be contaminated with Risk Group 2 or Risk Group 3 infectious biological agents pathogenic to otherwise healthy humans (clinical specimens from presumed healthy individuals do not meet this definition)
- Culturing/propagating unknown or uncharacterized microorganisms
- Analysis of specimens (i.e. blood, tissues, saliva, feces) from Old World monkeys (macaques, baboons)
- Use of a virus (i.e. EBV, SV40) to immortalize cell lines or use of immortalized cell lines capable of producing infectious virus(es)
- Use of acute biological toxins ($LD_{50} \leq 100 \mu\text{g/kg}$ body weight) or export-controlled toxins
- Experiments involving recombinant nucleic acid molecules (i.e. viral vectors, genetically-modified organisms)
- Experiments involving synthetic nucleic acids if they are designed to integrate into DNA, are replication competent or encode for a toxin with an $LD_{50} \leq 100 \text{ ng/kg}$ body weight
- Experiments using recombinant or synthetic nucleic acid molecules, or DNA or RNA derived from such molecules, in human research subjects (i.e. human gene therapy)
- Work that requires federal or state permits/notifications pertaining to regulated infectious materials (plant, animal, human pathogens), vectors or genetically modified organisms

Work involving biohazards shall be registered by logging into [Gator TRACS](#) and completing the Biohazard Project Registration. If you need assistance with the registration forms, please contact bsu@ehs.ufl.edu or call 352-392-1591.

Control of Biohazards

Once the hazards have been identified, control measures must be implemented and serve to minimize, or in some cases eliminate, the risk of working with biohazards. Risk mitigation is the final step in the risk assessment process. The hierarchy of controls (Figure 1) consists of five levels of action to reduce or remove hazards and can be applied to work with biohazards. The five levels are discussed in more detail in the following sections.

Figure 1: Hierarchy of Controls (NIOSH)



Elimination

Elimination completely removes the hazard. Elimination is often not feasible in the case of biohazards since the research typically relies on the use of the biohazard in experiments.

Substitution

Whenever possible, substitute less hazardous agents in place of their more hazardous counterparts. An example would be to use an attenuated strain of bacteria in experiments instead of using a more virulent/pathogenic strain.

Engineering Controls

Engineering controls are used to contain or remove biohazards, monitor critical physical parameters, or provide specific service. These include, but are not limited to, biological safety cabinets (BSCs), enclosed primary and secondary transport containers, directional airflow indicators, safety centrifuge cups, micro-isolator tops on animal cages, self-sheathing needles and sharps containers.

Biosafety Cabinets

Biological safety cabinets, also referred to as biosafety cabinets or BSCs, are the primary means of containment for working safely with infectious microorganisms.

Three kinds of BSCs designated as Class I, II, and III, have been developed to meet varying research and clinical needs. Most BSCs use high efficiency particulate air (HEPA) filters in their exhaust and/or supply systems. They provide product, personnel and environmental protection. The types of BSCs commonly used in laboratories are described in detail in [Appendix A of the BMBL: Primary Containment for Biohazards: Selection, Installation and Use of Biological Safety Cabinets](#). The most common type of BSC used at UF is a Class II Type A2 BSC.

Operations Within a Class II BSC:

- The BSC must be located:
 1. Away from the entry to the laboratory and from the laboratory traffic.
 2. Adequate clearance must be provided around, and 12-14" above, the BSC for easy access and to provide for accurate air velocity measurement.
 3. Away from open windows, portable fans, laboratory equipment that creates air movements and fume hoods.
- Good microbiological techniques (see [Principles of Good Microbiological Practice](#) handout) shall always be used when working in a BSC.
- Only materials and equipment required for the immediate work shall be placed in a BSC so as not to disrupt the airflow
- Frequent inward/outward movement needed to place objects in biohazard collection containers outside the BSC is disruptive to the integrity of the cabinet air barrier and can compromise both personal and product protection. Waste shall be collected inside the BSC. Horizontal pipette discard trays containing a disinfectant (e.g., bleach) are recommended for use inside the BSC
- Best practices recommend keeping clean materials at least 12 inches away from aerosol-generating activities will minimize the potential for cross-contamination.
- The general workflow shall be from clean to contaminated ("dirty"). Materials and supplies shall be placed in such a way as to limit the movement of dirty items over clean ones.
- Work at least 4" back from the front edge and never cover the front grill.
- When possible, open containers (tubes, bottles) shall be held at an angle to prevent contamination. Investigators working with Petri dishes and tissue culture plates shall hold the lid above the open sterile surface to minimize direct impact of downward air. Items shall be recapped or covered as soon as possible.
- The use of [open flames in BSCs](#) is heavily discouraged and almost always unnecessary.
- Aspirator bottles or suction flasks must contain adequate disinfectant (i.e., 100% bleach) such that liquid waste is inactivated as it is collected. An in-line hydrophobic HEPA filter shall be placed between the flask and the vacuum line to protect the central vacuum system and personnel who service the equipment.

- Ultraviolet (UV) lamps are not required or necessary in a BSC.
 1. If installed, UV lamps must be cleaned and checked periodically with a UV meter to confirm the appropriate wavelength is being emitted.
 2. UV lamps must be turned off when the room is occupied to protect eyes and reduce skin exposure.
 3. The sash on the BSC must be closed when operating the UV lamp.
- [Biological spills](#) in the BSC must be handled immediately. The [Handling Biological Spills](#) handout should be posted in the lab for easy reference.
- The BSC must be professionally certified per NSF/ANSI 49-2019 standards when it is used to handle/manipulate infectious and potentially infectious material. BSCs must be certified:
 1. After initial installation
 2. Annually thereafter
 3. After the BSC is relocated or repaired
 4. The BSC certification will include:
 - a. HEPA filter leak tests
 - i. Smoke delivered through T fitting to the middle or middle back of the work surface
 - ii. Scan rate no faster than 2inches/second in slightly overlapping strokes
 - iii. Includes entire periphery of the filter, along the bond between the filter pack and the frame, and around the seal between the filter pack and the device
 - iv. Both the cabinet supplying HEPA and the exhaust HEPA will be tested
 - b. Downflow air velocity measurement
 - i. The air measurement probe shall be held rigidly in a freestanding fixture that permits accurate positioning and does not distort the airflow pattern (ring-stand and clamp).
 - ii. measured at multiple points across the workspace, using equal points in the horizontal plane 4 in (10 cm) above the bottom edge of the window frame
 - c. Inflow air velocity measurement
 - d. Airflow Smoke Patterns tests
 - i. Down-flow
 - ii. View Screen retention
 - iii. Work opening edge retention
 - iv. Sash window seal
 - e. Confirmation of alarms function

Horizontal Laminar Flow Clean Benches

These are not BSCs! Horizontal laminar flow clean benches discharge HEPA-filtered air across the work surface and toward the user. These devices only provide product protection. They can be used for certain clean activities, such as the dust-free assembly of sterile equipment or electronic devices. These benches shall never be used when handling cell culture materials or drug formulations, or when manipulating potentially infectious materials. The worker can be exposed to materials (including proteinaceous antigens) being manipulated on the clean bench, which may cause hypersensitivity.

Horizontal clean air benches shall never be used as a substitute for a biological safety cabinet in research, biomedical or veterinary laboratories and/or applications.

Vertical Laminar Flow Clean Benches

These are not BSCs! They may be useful, for example, in hospital pharmacies when a clean area is needed for preparation of intravenous drugs. While these units generally have a sash, the air is usually discharged into the room under the sash, resulting in the same potential problems as the horizontal laminar flow clean benches.

Administrative Controls

Administrative controls are put in place to minimize exposure to hazards. Administrative controls include items such as training, standard operating procedures (SOPs), laboratory signage and documentation, emergency preparedness and medical monitoring.

Training

Education and training are essential for ensuring laboratory personnel have learned about the hazards in the lab and how to appropriately manage the hazards. UF EH&S offers a variety of [training courses](#), most of which are available through [myTraining](#). Courses specific to laboratories working with biological hazards include Bloodborne Pathogens (EHS850G), Biomedical Waste (EHS851), Shipping and Transport of Biological Materials (EHS852; must email bsa@ehs.ufl.edu to enroll in this course) and General Biosafety Training (EHS853).

Standard Operating Procedures (SOPs)

A standard operating procedure (SOP) is a set of written instructions for performing experiments or processes that involve hazards (biological, chemical, radiation, physical, etc.) SOPs are lab-specific and it is the PI's responsibility to ensure that lab staff receive training on the SOPs applicable to their work. Additional information regarding SOPs, including a guidance manual and templates, can be found by clicking [here](#).

Medical Monitoring

The [EH&S Occupational Medicine Program](#) provides a variety of services to meet the health and safety needs of UF employees and students. In collaboration with other groups on campus, the Occupational Medicine program provides medical monitoring, testing services and vaccinations required for work with various biohazards. [BioPath](#) is the medical monitoring program designed for individuals working in a BSL-3 or ABSL-3 laboratory at UF.

Personal Protective Equipment (PPE)

Personal protective equipment (PPE) refers "equipment worn to minimize exposure to hazards that cause serious workplace injuries and illnesses." Examples of PPE include coveralls, lab coats, gloves, face shields, safety glasses, goggles, safety shoes and respirators. Details about PPE requirements at UF and types of PPE commonly used in laboratories can be found by clicking [here](#). If PPE above the minimal requirements is needed for work with biohazards, it will be relayed to the laboratory as part of the Biohazard Project Registration approval process.

Biohazard Incidents

While control measures can minimize the risk of exposure to biohazards, accidents do happen.

In the event of a life-threatening emergency, always call 911.

Biological Spills

Biological spills must be handled promptly, and personnel shall be familiar with spill response procedures. All laboratories working with biological materials shall have a [Biological Spill Kit](#) assembled and the [Handling Biological Spills](#) handout shall be posted or kept with the spill kit. For extremely large or high hazard biological spills, contact the Biosafety Office (352-392-1591) for assistance.

Biological Exposures

Exposures may occur through percutaneous injuries (cut/puncture with sharp objects such as needles or broken glass or bites from infected animals), splashes to mucous membranes, contact of biological materials with non-intact skin, inhalation and ingestion. Percutaneous injuries and non-intact skin exposures should be washed with soap and water as soon as possible. For mucous membrane exposure, use the nearest eyewash station to flush mouth/nose/eyes.

Human blood/OPIM exposures should be reported to the Needlestick Hotline at 352-265-2727. This number is answered 24 hours a day, 7 days a week. After you have been evaluated/treated, contact AmeriSys (800-455-2079) to report the incident.

For non-BBP biological exposures, employees should contact AmeriSys (800-455-2079) to report the incident and obtain authorization for medical treatment, if needed. Students should seek care through their personal physician or preferred medical provider.

Incident Reporting

All biological spills, exposures (overt or potential), accidents and injuries involving biological materials must be documented and reported to EH&S using the [Injury/Incident Report Form](#). Incidents must be reported as soon as possible and within 24 hours as some types of incidents (i.e., those involving reportable diseases, regulated material or certain types of recombinant/synthetic nucleic acids) require additional reporting to local, state and/or federal agencies.

Laboratory Safety Surveys

The Biosafety Office performs safety surveys in laboratories, greenhouses and animal facilities working with biohazards on a periodic basis. The purpose of these surveys is several fold:

- as part of the risk assessment process to identify and correct potential hazards,
- to facilitate compliance with local, state, and federal requirements, and
- to provide guidance and information on relevant biosafety issues to Principal Investigators, staff, and students.

Safety surveys are generally scheduled with the laboratory, but unannounced surveys may occur in some instances. Routine self-audits are also recommended. The checklist used for the survey depends on the type of research being performed and the biosafety level(s) assigned to the work.

- Facilities working with infectious or potentially infectious materials are generally inspected based on the requirements specified in [BMBL 6th edition](#). Specific requirements for BSL-1 through BSL-4 are outlined in Section IV, Laboratory Biosafety Level Criteria. [Checklists](#) based on these criteria for BSL-1, BSL-2, and BSL-3 facilities are available online. There are no BSL-4 facilities at UF.
- Facilities working with infectious or potentially infectious materials in animals are inspected based on the requirements specified in [BMBL 6th edition](#). Specific requirements for ABSL-1 through ABSL-4 are outlined in Section V, Vertebrate Animal Biosafety Level Criteria. [Checklists](#) based on these criteria for ABSL-1, ABSL-2, and ABSL-3 facilities are available online. There are no ABSL-4 facilities at UF.
- Laboratories that work with biological toxins are inspected for compliance with UF policies, as described in the [Acute Toxin Standard Operating Procedures template](#), and federal guidelines detailed in Appendix I of the [BMBL 6th edition](#).
- Facilities working with recombinant or synthetic nucleic acids are inspected for compliance with the appropriate appendices of the NIH Guidelines.
 1. For lab-scale recombinant or synthetic nucleic acid molecules work, [Appendix G](#).
 2. For Large Scale (>10L) recombinant or synthetic nucleic acid molecules work, [Appendix K](#).
 3. For recombinant or synthetic nucleic acid molecule work involving plants, [Appendix L](#) (note that the biosafety levels described by the NIH in Appendix L are referred to as BSL-1P through BSL-4P).
 4. For recombinant or synthetic nucleic acid molecules work involving animals [Appendix M](#) (note that the biosafety levels described by the NIH in Appendix Q are referred to as BSL1-N through BSL4-N).

Permits

Areas where biological agents regulated by state or federal permits are used, stored or planted/released are inspected based upon the conditions specified by the permit itself, as well as best practices listed by the agency issuing the permit. Common permitting agencies for biological material are the [State of Florida Division of Plant Industry](#), [USDA Biotechnology Regulatory Services](#) (BRS), [USDA/APHIS Import/Export Services](#), [CDC Etiologic Agent Import Permit Program](#), [FDA Center for Veterinary Medicine](#) (CVM), [FDA Center for Biologics Evaluation and Research](#) (CBER) and the [EPA](#). Additional information can be found on the [Permits and Documentation](#) page.

Sterilization, Disinfection and Decontamination

Proper cleaning and disinfection or sterilization of contaminated surfaces and equipment is an essential step in mitigating the potential for transmitting infection to personnel.

Decontamination

A process or treatment that renders a device, instrument or work surface safe to handle. Sterilization and disinfection are forms of decontamination. It is important to note that a prerequisite for many decontamination procedures is adequate pre-cleaning of the item to be decontaminated to remove any gross contamination that will interfere with the disinfectant's action.

Sterilization

A process that destroys or eliminates all forms of microbial life, including spores. Sterilization can be achieved through a variety of means, including heat, chemicals, irradiation, high pressure and filtration. At UF, steam sterilization (autoclaving) is the most commonly used method of sterilization. Autoclaving can be used to inactivate biological waste as well as to sterilize laboratory supplies, surgical instruments and equipment.

Autoclaves

The rules governing the use and testing of autoclaves are based on [Chapter 64E-16.007 of the Florida Administrative Code](#). Autoclaves shall be tested before being placed into service and then periodically for effectiveness:

- Every 40 hours of sterilization phase use (required for autoclaves that are used to inactivate human or non-human primate blood, tissues, clinical samples, or human pathogens.)
- Every 6 months (required for autoclaves that are used to sterilize other material.)

Information regarding autoclave operation, testing, record-keeping and training can be found at: <https://www.ehs.ufl.edu/departments/research-safety-services/biosafety/autoclaves/>.

Disinfection

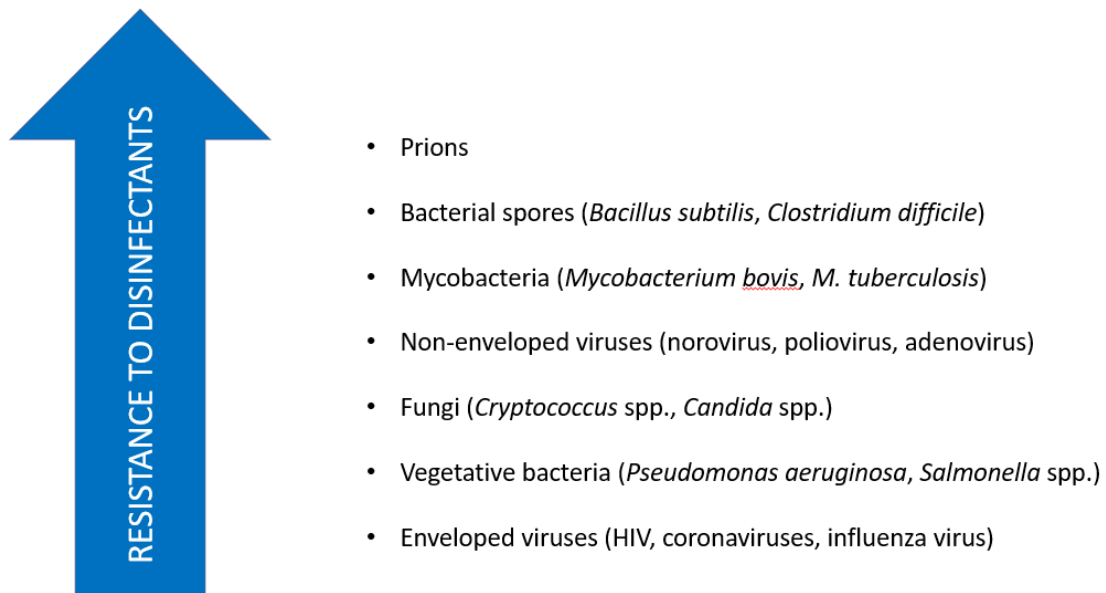
A process that eliminates many or all pathogenic microorganisms, excluding bacterial spores, on work surfaces and equipment. Liquid disinfectants are typically used for surface decontamination and can also be used to decontaminate liquid wastes provided there is adequate concentration and contact time. The effectiveness of a liquid disinfectant is dependent on a variety of factors, including:

- Nature and number of contaminating microorganisms
- Organic load (e.g. soil, blood, feces)
- Microbial load and type of organism
- Condition of surfaces to be disinfected (porous vs nonporous)
- Concentration, pH, temperature and contact time of the disinfectant

Chemical disinfectants range in activity from high-level disinfectants that can be used to decontaminate spills of infectious agents to low-level disinfectants that can be used for general housekeeping purposes.

Figure 2 shows resistance of selected organisms to disinfectants with prions being the most difficult to decontaminate. Generally, laboratories should choose a disinfectant that has a broad range of activity. The Biosafety Office recommends the use of a disinfectant with efficacy claims against *Mycobacterium tuberculosis* (tuberculocidal disinfectant). A list of such disinfectants is available from the EPA (see [List B: Antimicrobial Products Registered with EPA for Claims Against Mycobacterium tuberculosis \(TB\)](#)).

Figure 2: Resistance to Disinfectants



Characteristics of Selected Disinfectants

There are a variety of disinfectant types (alcohols, iodophors, chlorinated compounds, quarternary ammonium compounds, etc.) and when choosing which disinfectant to use, one should consider the organism/virus being targeted, the item to be disinfected, hazardous properties of the disinfectant and cost and ease of use of the disinfectant. Always consult manufacturer instructions for use and the Safety Data Sheets (SDS). Table 3 describes properties of some commonly used disinfectants.

Table 3: Properties of Commonly Used Disinfectants

Class of Disinfectant	Mode of Action	Advantages	Disadvantages	Hazards
Alcohols	Denatures proteins causing membrane damage and cell lysis	Rapidly bactericidal against vegetative bacteria Leaves no residue Inexpensive	Evaporates before required contact time Not effective against spores Inactivated by organic matter Variable efficacy against non-enveloped viruses	Flammable
Chlorine & chlorine compounds (e.g. bleach)	Denatures proteins	Fast acting Inexpensive Effective against a	Corrosive to metals Inactivated by organic matter	Potential to generate chlorine gas if mixed with other disinfectants

		broad spectrum of microorganisms	Must be made fresh daily	Do not autoclave Skin and eye irritant
Peroxygen compounds (e.g. hydrogen peroxide)	Produce hydroxyl free radicals that can attack membrane lipids, DNA and other essential cell components	Fast acting Environmentally friendly Potent and broad-spectrum antimicrobial activity Low toxicity at lower concentrations	May be corrosive to some metals (lead, copper, brass, zinc) Inactivated by organic matter	Skin and eye irritant
Phenolics	Disrupts cell wall and precipitates proteins	Effective against a broad spectrum of microorganisms Effective in presence of organic matter Non-corrosive	Leaves residual film on surfaces	Skin and eye irritant
Quarternary ammonium compounds ("QUATS" or QAC)	Denature proteins and disrupt cell membrane	Readily available Effective against most vegetative bacteria, fungi and enveloped viruses	Inactivated by organic matter Not effective against spores Poor efficacy against Gram positive bacteria	Skin and eye irritant

Equipment Decontamination

Laboratory equipment (refrigerators, freezers, biosafety cabinets, incubators, centrifuges, etc.) must be decontaminated prior to moving, disposing or repairing. The equipment must be decontaminated in a manner appropriate for the hazards/potential hazards it was used with. Complete the [EH&S Equipment Decontamination Form](#) for all lab equipment.

Space Decontamination

Space decontamination is a highly specialized activity that must be performed by individuals with proper training and expertise. Please contact the Biosafety Office for more information.

Training

The Biosafety Office provides several training courses that are applicable to personnel working in biological laboratories. These courses are briefly described below, and most can be accessed through

the [myTraining](#) platform. It is important to note that the PI is responsible for providing hazard-specific training to all personnel as indicated in the LATCH risk assessment and the PI Certification for biohazard projects. In-person training may be available for specific situations – please contact the Biosafety Office to discuss your training needs. For a full list of training courses offered by EH&S, click [here](#).

Autoclave Training (EHS871)

Autoclave training is provided **in-person** at the autoclave and typically lasts about one hour. Training is scheduled by the facility or lab as needed. Due to room-size limitations and the need to maximize efficiency, sessions will be scheduled for 3-6 individuals at a time. Contact the Biosafety Office (392-1591 or bsa@ehs.ufl.edu) to schedule autoclave training.

Biomedical Waste (EHS851)

Biomedical Waste training is required for all individuals who generate and/or package biomedical waste. Training is required upon hire or when first assuming duties associated with biomedical waste and annually (within 365 days) thereafter per State of Florida Department of Health regulations. Additional information on biohazardous waste disposal can be found at:

<https://www.ehs.ufl.edu/departments/research-safety-services/biosafety/biohazardous-waste/>.

Bloodborne Pathogens (EHS850G)

Bloodborne Pathogens training is required for all individuals with reasonably anticipated occupational exposure to human blood and other potentially infectious materials (human cells, tissues, certain body fluids). Training is required at the time of initial assignment to tasks where occupational exposure may take place and annually (within 365 days) thereafter. See the [Bloodborne Pathogen Program](#) page for the Exposure Control plan and other information.

General Biosafety (EHS853)

General Biosafety is required for all individuals working with, or supervising work with, recombinant/synthetic nucleic acids, infectious agents and acute biological toxins. At this time, training must be completed once.

Shipping and Transport of Biological Materials (EHS852)

The shipping and transport of dangerous goods is highly regulated, and many biological materials fall into this category. All individuals involved in the transport or preparation of dangerous goods must be trained. To register for this course, please email your name and UF ID number to bsa@ehs.ufl.edu and request enrollment in the Shipping and Transport of Biological Materials course. Training is valid for 2 years. For additional information related to shipping biological materials, see

<https://www.ehs.ufl.edu/departments/research-safety-services/biosafety/shipping-and-transport/>.

Appendix

Resources

Biosafety in Microbiological and Biomedical Laboratories:

https://www.cdc.gov/labs/pdf/SF_19_308133-A_BMBL6_00-BOOK-WEB-final-3.pdf

CDC Etiologic Agent Import Permit Program

<https://www.cdc.gov/orr/ipp/index.htm>

Federal Select Agent Program

<https://www.selectagents.gov/>

Florida Department of Agriculture and Consumer Services, Division of Plant Industry

<https://www.fdacs.gov/Divisions-Offices/Plant-Industry>

Florida Department of Health Biomedical Waste Program

<https://www.floridahealth.gov/environmental-health/biomedical-waste/index.html>

Gator TRACS

<https://labcliq.com/l/ufl/>

NIH Guidelines for Research Involving Recombinant and Synthetic Nucleic Acid Molecules

https://osp.od.nih.gov/wp-content/uploads/2019_NIH_Guidelines.htm

Occupational Safety and Health Administration (OSHA) Bloodborne Pathogens Standard

<https://www.osha.gov/laws-regs/regulations/standardnumber/1910/1910.1030>

Pathogen Safety Data Sheets

<https://www.canada.ca/en/public-health/services/laboratory-biosafety-biosecurity/pathogen-safety-data-sheets-risk-assessment.html>

Risk Group Database (ABSA)

<https://my.absa.org/tiki-index.php?page=Riskgroups>

US Department of Transportation, Check the Box: Getting Started with Shipping Hazmat

<https://www.transportation.gov/check-the-box/getting-started-with-hazmat>

USDA APHIS Biotechnology Regulatory Service (BRS)

<https://www.aphis.usda.gov/aphis/ourfocus/biotechnology>

USDA APHIS Organisms and Vectors Guidance & Permitting

https://www.aphis.usda.gov/aphis/ourfocus/animalhealth/animal-and-animal-product-import-information/organisms-vectors/ct_organisms_and_vectors

USDA APHIS Plant Health Permits

<https://www.aphis.usda.gov/aphis/ourfocus/planthealth/import-information/permits>

USG Policy for Institutional Oversight of Life Sciences Dual Use Research of Concern

<https://www.phe.gov/s3/dualuse/documents/durc-policy.pdf>

University of Florida Environmental Health & Safety

<https://www.ehs.ufl.edu/>

University of Florida Institutional Biosafety Committee (IBC)

<http://ibc.research.ufl.edu/>

World Health Organization Laboratory Biosafety Manual (4th edition)

<https://apps.who.int/iris/rest/bitstreams/1323419/retrieve>